Establishment of guayule plants in a limed bark medium at low phosphate levels

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Seedlings of guayule (*Parthenium argentatum* Gray) were successfully grown on a milled and composted pine bark medium. Liming and saturation of the medium was carried out for a 4-week period, prior to the addition of a VAM fungal inoculum and guayule seeds. This was done to permit an increase and stabilization of pH. Rock phosphate was incorporated into the medium at levels of 1.5, 3, 6 and 12 mg dm^{-3}, while other nutrients were provided by a modified Hoagland's solution (lacking phosphate) with a nitrogen level of 70 mg dm^{-3}. Although poor seedling growth was recorded, it is suggested that seedlings may be pre-adapted to transplantation in a potentially hostile environment.

Saailinge van guayule (*Parthenium argentatum* Gray) is suksesvol op 'n gemaalde en gekomposteerde dennebas-medium gekweek. Kalsifering en versadiging van die medium is bewerkstellig vir 4 weke voor die toediening van 'n VAM-fungus-inokulum en guayule-saad. Hierdie stap is ingesluit om 'n toename en stabilisering van die pH te bewerkstellig. Rotsfosfaat is teen vlakke van 1.5, 3, 6 en 12 mg dm^{-3} in die medium geïnkorporeer. Ander voedingstowwe (fosfaat uitgeslote) is verskaf deur 'n gemodifiseerde Hoagland-oplossing met 'n stikstofvlak van 70 mg dm^{-3}. Alhoewel saailinge swak gegroei het, word daar nogtans voorgestel dat saailinge vooraf gekondisioneer word vir oorplanting in 'n potensiële omgewing.

Keywords: Mycorrhiza, *Parthenium argentatum*, seedling establishment

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Introduction

Guayule (*Parthenium argentatum* Gray), is a semi-desert shrub, which has the ability to produce economically viable amounts of natural rubber. The cultivation of guayule from seed is plagued by problems of high seed expense, low seed germinability and poor seedling vigour. A more practical and successful method of cultivation may be container-grown transplants. Since vesicular arbuscular mycorrhizal (VAM) inocula in soilless organic media have been used to produce inoculated container-grown citrus seedlings of high survival (Graham & Timmer 1985), the application of this approach to guayule seedlings might have merit. Indeed, the VAM fungus *Glomus intraradices* Schenck & Smith is associated with guayule in nature (Bloss 1980) and has been shown to enhance initial seedling vigour and post-transplant survival rates (Bloss & Pfeiffer 1981, 1984).

The availability of phosphate to the mycorrhizal complex is considered to be the single most important factor governing mycorrhizal establishment (Hayman 1983). The physical and chemical properties of a soilless organic medium, such as pine bark, differ markedly from those of soil. The ability of bark to retain phosphate is virtually zero and, as a result, phosphate is supplied to container-grown crops either as a soluble inorganic form in a nutrient solution, or as a solid fertilizer which is incorporated into the medium (Biermann & Linderman 1983).

It has further been shown that relatively high levels of fungal infections were achieved when phosphate levels within the medium were maintained at a constant low level throughout seedling development with the use of slow-release phosphate sources such as rock phosphate (Graham & Timmer 1985).

The objectives of this study were to investigate the release of phosphate in a limed pine bark medium and to observe the effect on both plant growth and fungal infection.

Materials and Methods

Growth medium

An organic medium consisting of milled and composted pine bark, was sieved through a 5-mm mesh sieve before being autoclaved at 121°C for 1 h. Dolomite and calcitic lime were added to the autoclaved bark at a rate of 4 g dm^{-3} and the medium was maintained in a saturated condition over a 4-week period to raise the pH to approximately 5.8. The limed bark was then divided into four lots, to which rock phosphate (Langebaan Raw Phosphate, total P = 12.6%, Ca = 29%) was added at levels of 1.5, 3, 6 and 12 mg dm^{-3}.

The medium was then potted into high-density polystyrene speedling trays (cube volume = 50 cm^3; bulk density of bark = 0.48 g cm^{-3}). The trays were transferred to an open shadehouse under mist, and watered once a day for approximately 15 min by an overhead mist-spraying system.

Seedling growth

Guayule seed (cultivar W10; U.S. strain A.48118) was initially pretreated for 2 h in a hormone solution consisting of a gibberellic acid solution, made up by diluting 0.9 cm^3 of the active ingredients (gibberellins 4

and 7 — 2% V/V) in distilled water, plus two drops of Tween 20. The treated seeds were air-dried before being sown at a rate of three seeds per speedling cube. Two weeks after seeding, the seedlings were thinned out to one seedling per cube and applications of modified Hoagland's solution begun. In order to examine the influence of an absence of phosphate on plant growth, potassium dihydrogen phosphate was omitted from this watering solution. However, since calcium was a constituent of rock phosphate, calcium nitrate was also omitted from the watering solution. Potassium nitrate was therefore the sole nitrogen source in the modified Hoagland's solution at a level of 70 mg cm^3.

Mycorrhizal inoculation

An inoculum of *Glomus intraradices* consisting of spores, chopped roots and potting medium, was obtained from Sudan grass [*Sorghum vulgare* var. *sudanese* (Piper) Hitchc.] grown in pot culture. The isolate was kindly provided by Dr H.E. Bloss. This was added to the bark medium at a rate of 2.5 g dm^{-3}. Pine bark medium without inoculum was used for the controls. Two sets of mychorriza-free guayule seedlings were set up as controls. One was fertilized with normal, full-strength Hoagland's solution while the other control received Hoagland's solution without phosphate.

Mycorrhizal infection

Infected root systems were cut into 0.5- to 1-cm segments, and the pieces were cleared and stained according to the methods of Brundrett *et al.* (1984). Twenty-five pieces per treatment were spread out in a Petri dish, viewed at 20× under a dissecting microscope and the percentage of the length of root segments containing hyphae estimated according to the procedure described by Biermann & Linderman (1981). Four readings per root sample were used to obtain a mean value.

Phosphate determination

Available phosphate was determined at weekly intervals for each of the concentrations tested. Each cube was placed in a 100-cm^3 beaker and approximately 15 cm^3 of distilled water was added until the bark just became saturated. The samples were allowed to equilibrate for an hour before being stirred. After an additional half hour the pH was recorded before the free water was removed from the samples by sucessive filtration through Watman No. 1 filter paper and a 0.45-μm Millipore membrane filter. Each sample yielded approximately 12 cm^3 of extract and these were then analysed for soluble phosphate. Ten millilitres of extract were pipetted into a 50-cm^3 volumetric flask followed by 4 cm^3 of a working reagent (Mackereth *et al.* 1978), and the flask made up to volume with distilled water. The preparations were left for 10 min for the colour reaction to develop, after which the absorbance was read at 880 nm.

A standard curve was prepared weekly from a stock solution of 0.1 g dm^{-3} of anhydrous KH_2PO_4 in distilled water. Following a 100-fold dilution, aliquots of 0.5, 1, 2.5, 7.5 and 10 cm^3 were pipetted into 50-cm^3 volumetric flasks, developed with 4 cm^3 of the working reagent (as above) and made-up to volume, giving phosphate concentrations of 10, 20, 50, 100, 150 and 200 μg dm^{-3}.

Phosphate levels were also determined for unlimed bark.

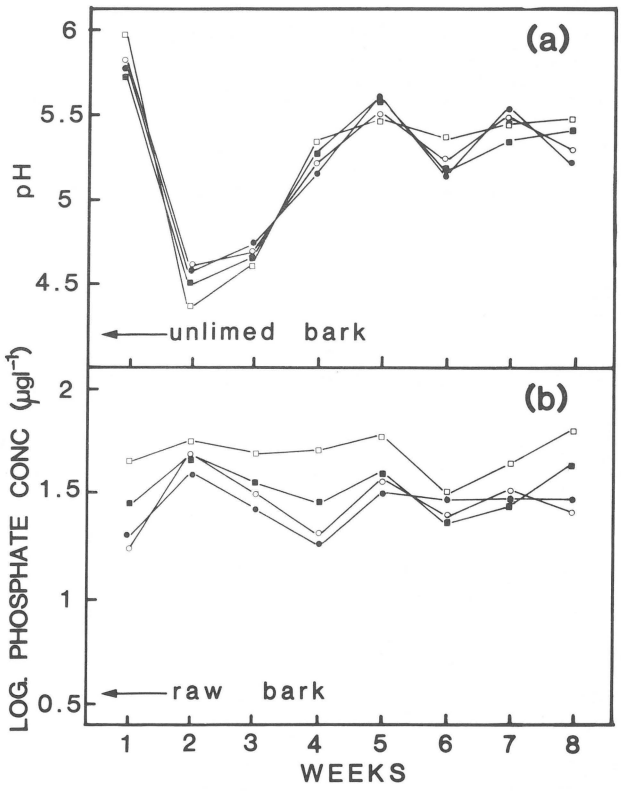

Figure 1 Changes in the pH (a) and the availability of soluble phosphate (b) of a limed bark medium amended with four levels of rock phosphate: (●) 1.5, (○) 3, (■) 6 and (□) 12 mg cm^{-3}.

Growth measurements

After 12 weeks growth in speedling trays, the plants were harvested. Replicates of six plants per treatment were used for growth measurements. Leaf area was measured on a Li-COR model Li3100 area meter, while dry mass was determined after drying for 48 h at 80°C.

Results

Over the first 8 weeks of the experimental period the pH of the limed bark showed fluctuations following initial treatment (Figure 1a). Immediately after liming, the pH of the bark medium rose to approximately 5.75, as compared to the unlimed bark medium of pH 4.2. One week after liming, the pH of the medium had dropped to 4.5 but gradually rose to pH 5.5 over the next 3 weeks. Minor fluctuations in the pH were observed over the remaining few weeks. The pH values for the different rock phosphate-amended bark treatments differed slightly. The pH of the media receiving the highest rock phosphate supplement did not fluctuate as much as those receiving lower concentrations.

The available phosphate recorded over the same

period tended to remain fairly constant. Apart from some exceptions in the last 3 weeks of monitoring, media receiving increasing rock phosphate concentrations, tended to have higher levels of available phosphate (Figure 1b). No clear relationship was apparent between available phosphate and the pH of the bark medium, although the highest concentration of added rock phosphate tended to show a more constant level of available phosphate than did the lower additions, which showed a more variable pattern.

The growth of guayule seedlings, as measured by dry mass, indicated that all the treatments receiving rock phosphate grew poorly, the statistical differences between the treated plants and those receiving no phosphate being negligible (Figure 2). Deficiency symptoms, such as yellowing of leaf edges and, in extreme cases, leaf edge necrosis were observed. Total leaf areas were also very low in the treated plants, although the leaf areas of controls not receiving phosphate were not statistically different from those of the rock phosphate-treated plants (Figure 3). These results are in sharp contrast to those of plants grown on a full-strength Hoagland's solution where statistically significant increases in both total dry weight and leaf area were evident (Figures 2 and 3).

V-A mycorrhizae were present in all the rock phosphate-treated plants although the extent of infection varied within treatments. No colonization was observed in control seedlings, grown without inoculum, in open-pot culture. The highest level of infection (30.6%) was recorded for plants receiving the second lowest phosphate level (i.e. 3 mg l^{-1}), while the lowest rate of infection occured in plants grown at phosphate concentrations of 1.5 mg l^{-1} (Figure 4). Microscopic observations revealed that the VAM fungus to be in a mature phase of growth, consisting almost entirely of vesicles.

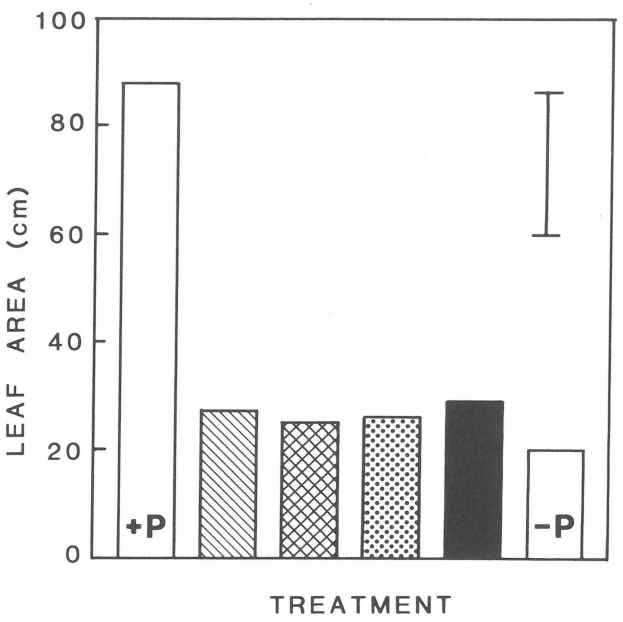

Figure 3 Leaf area of guayule seedlings grown in a limed bark medium at rock phosphate levels of: (▨) 1.5, (▩) 3, (⊡) 6 and (■) 12 mg cm^{-3} in the presence of VAM fungus. +P and –P represent the growth of non-mycorrhizal controls in a Hoagland's medium with and without phosphate respectively. *LSD* ($P = 0.05$) within treatments indicated by bar.

Discussion

Milled and composted pine bark can be regarded as an

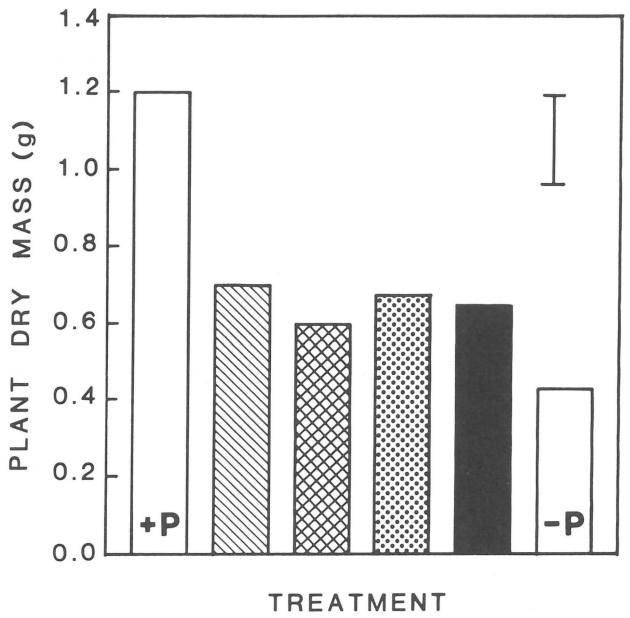

Figure 2 Dry mass of guayule seedlings grown in a limed bark medium at rock phosphate levels of: (▨) 1.5, (▩) 3, (⊡) 6 and (■) 12 mg cm^{-3} in the presence of VAM fungus. +P and –P represent the growth of non-mycorrhizal controls in a Hoagland's medium with and without phosphate respectively. *LSD* ($P = 0.05$) within treatments indicated by bar.

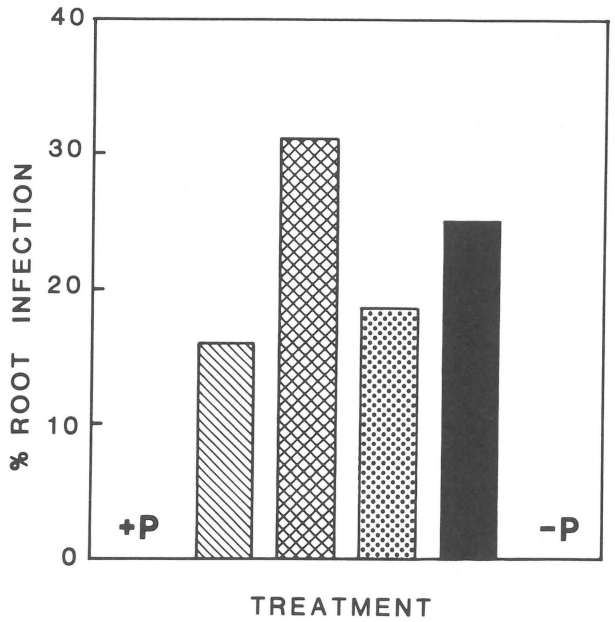

Figure 4 Percentage mycorrhizal root infection of guayule seedlings grown in a limed bark medium at rock phosphate levels of: (▨) 1.5, (▩) 3, (⊡) 6 and (■) 12 mg cm^{-3}. +P and –P indicate the presence or absence respectively of phosphate in a Hoagland's medium given to uninfected controls.

inert supportive medium for the growth of seedlings. All nutrients required for plant growth, and for the VAM fungus to establish infection, must be supplied either as a liquid fertilizer or added to the medium in the form of a slow-release fertilizer since phosphates and nitrates are rapidly leached out on irrigation (Foster *et al.* 1983; Biermann & Linderman 1983).

However, raw bark is highly acidic and 4 weeks were required for the pH of the medium to stabilize at approximately pH 5.4. The solubility of rock phosphate is dependent on pH (Mosse *et al.* 1976) and in the present experiment available phosphate release remained fairly constant over the initial period of high pH change. It would appear that as the concentration of rock phosphate within the medium rises, so does the buffering capacity of the medium with a resultant steadying in the release of available phosphate.

The pH of the growing medium also exerts a selective quality as to the infectivity of different types of VAM fungi. Fine endophytes exclusively colonize roots at low pH while coarse endophytes, such as *Glomus* spp., prefer higher pH. Ultimately, in alkaline soils, roots tend to be colonized exclusively by these endophytes (Wang *et al.* 1985).

Phosphate concentrations of between 4 and 5 mg dm^{-3} have been suggested to be critical levels for mycorrhizal development in a soilless organic medium (Biermann & Linderman 1983), although concentrations as low as 0.3 mg dm^{-3} have also been found to initiate high levels of infection (Ojala & Jarrell 1980).

In the present study the highest application of rock phosphate (12 mg) was only able to provide phosphate at a level of 0.049 mg dm^{-3}, and a marked decline in plant growth was therefore to be expected. Phosphate concentrations lower than 0.1 mg dm^{-3} are regarded as being too low for adequate plant growth in the absence of VAM fungi (Ojala & Jarrell 1980; Howeler *et al.* 1981; Mosse 1973).

Twelve-week-old seedlings grown in pine bark medium, inoculated with VAM fungus, and irrigated with a Hoagland's solution containing KH_2PO_4 (which provided phosphate levels ranging from 0.031–0.31 mg dm^{-3}) were found to have levels of mycorrhizal infection ranging from 20–27% (Vietti 1987), which is not greatly different from the range of 15–30% recorded for seedlings provided with rock phosphate.

Although superior growth was recorded in this study for plants watered with full-strength Hoagland's solution and lacking both rock phosphate and VAM fungus, it is well known that mycorrhizal plants have an advantage over non-mycorrhizal plants as regards their ability to cope with transplant, moisture and salinity stress (Maronek *et al.* 1981). It might therefore be predicted that successful field transplantation of rock phosphate-supplemented seedlings would be possible because of the inherent advantages of mycorrhizal colonization and reduced aerial growth. In view of the approximately inverse relationship between the amount of labile phosphate in the soil and the plant growth response to VAM (Daft & Nicolson 1969) the use of a modified Hoagland's solution, and rock phosphate in a pine bark medium may pre-adapt seedlings transplanted to a potentially hostile environment.

Acknowledgements

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